Effect of Processing on the Amino Acid Composition and Nitrosamine Formation in Pork Belly Adipose Tissue

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The curing process does not alter the concentration of free amino acids in the adipose tissue of pork bellies. Frying of the adipose tissue leads to a decrease in the level of free amino acids; greater reductions were seen in the processed vs. untreated tissue. Analysis for nitrosamines in the fried samples indicate that dimethylnitrosamine and nitrosopyrrolidine formation is independent of free amino acid concentration. A study of the lean, adipose and intact tissue of country cured bacon indicates that frying generates up to a tenfold increase in free amino acids in the lean and intact tissue, while comparatively negligible changes are noted in the adipose.

The potential human health hazard posed by nitrosamines (NAs) was recognized as early as 1954 by Barnes and Magee. NAs are produced by the acid-catalyzed reaction of nitrite or nitrogen oxides with certain nitrogen-containing compounds. Amino acids, amines, and amides are examples of these compounds that are present as natural constituents of meats and other foodstuffs (Lijinsky et al., 1970; Ender and Ceh, 1971; Bills et al., 1973; Huxel et al., 1974). The formation of NAs has been reported in model systems in which amino acids are heated with sodium nitrite at elevated temperatures (~170 °C) similar to those attained when frying bacon (Ender and Ceh, 1971; Bills et al., 1973; Huxel et al., 1974; Gray and Dugan, 1973; Coleman, 1978).

Traditionally, nitrite and to a lesser extent nitrate have been used to prepare cured meats that are shelf-stable and possess desirable color and flavor characteristics. Although NAs are not found consistently in all cured meat products

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in which nitrite is an additive, nitrosopyrrolidine (NPYR) has been confirmed repeatedly in fried bacon (Sen et al., 1973; Fazio et al., 1973; Fiddler et al., 1974; Patterson et al., 1976; Havery et al., 1976; Gray, 1976). The formation of NPYR has been thought to occur by either of two mechanisms: decarboxylation of proline to pyrrolidine which is then nitrosated or the initial formation of nitrosoproline followed by decarboxylation. Recent studies, however, indicate that NPYR forms almost exclusively in the bacon adipose tissue rather than in the lean tissue where most amino acids are found (Fiddler et al., 1974; Mottram et al., 1977). Amino acid data, however, on pork belly adipose tissue are limited.

We determined amino acid concentrations of adipose tissue in matched pairs of processed and untreated bellies before and after frying and nitrosamine values from the corresponding samples after heating them with excess nitrite to ensure maximum NA formation. We excluded ascorbate from the cure solution because it reacts competitively with nitrite, which could reduce NA formation. We also analyzed "Country Cured" bacon, a commercial product, which contains no ascorbate and, therefore, closely resembles the bacon used in this study.

EXPERIMENTAL SECTION

a. Bacon Processing. Matched pairs of freshly skinned pork bellies were purchased from a local packing plant within 3 days of slaughter. One side of each pair was stored at 4 °C; the other side was pumped to 110% of green weight with a cure pickle consisting of 787 mL of H_2O, 147 g of NaCl, 50 g of sugar, 30 g of sodium tripolyphosphate, and 1.6 g of $NaNO_2$. The final target levels achieved prior to smoking were 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, and 120 ppm sodium nitrite. The bellies were then stored in polyethylene bags at 1 °C for 18–24 h prior to being processed into bacon. The smokehouse schedule was 1 h at 48.9 °C (120 °F) and 3–4 h at 58.3 °C (137 °F) at 50% relative humidity, with a medium level of smoke produced from hardwood sawdust.

b. Sample Preparation. Adipose and lean tissues from entire bellies were separated manually from green bellies, laboratory processed bacon, and commerical dry-cured bacon. Separated and intact tissues were ground twice and mixed thoroughly to attain sample homogeneity and then stored at −20 °C until used. All samples that were heated had 1000 ppm $NaNO_2$ added; they were then fried in a preheated Presto Teflon-coated electric frying pan (Model PC4AT) for 6 min at 177 °C (350 °F). Intact and adipose tissues were cooked normally, but in order to attain the same temperatures the lean tissue was fried in a hydrogenated vegetable oil.

METHODS OF ANALYSIS

a. Amino Acids. The extraction procedure used was similar to that described by Lakritz et al. (1974). The acid–neutral amino acids were separated on a Spherix XX8-60-O resin in a Phoenix Model M-7800 amino acid analyzer, by the accelerated system of Spackman et al. (1958), with sodium citrate buffers.

b. Volatile Nitrosamines. The NAs in the cooked samples were determined by the isolation procedure of Fazio et al. (1971) as modified by Pensabene et al. (1974), with a silica gel acidified Florisil chromatographic column. Samples were quantitated by a gas–liquid chromatograph interfaced with a thermal energy analyzer (Model 502) under conditions similar to those reported by Fine et al. (1975). NAs were confirmed by a GLC–high-resolution mass spectrometer (Model DuPont 21-492) (Pensabene et al., 1974).

Table I. Amino Acid Composition of Pork Belly Adipose Tissue

amino acids	concn,[a] μM/100 g	
	untreated	processed
Asp	2.3 ± 0.5	2.5 ± 0.6
Thr–Ser[b]	44.9 ± 5.1	57.1 ± 4.8
Glu	11.0 ± 3.3	27.8 ± 15.8
Pro	8.0 ± 0.8	12.1 ± 1.6
Gly	40.6 ± 4.8	57.2 ± 8.1
Ala	46.2 ± 12.2	41.3 ± 8.1
Val	11.1 ± 1.8	12.8 ± 3.2
Met	1.9 ± 0.6	1.6 ± 0.5
Ile	4.3 ± 0.6	5.6 ± 1.6
Leu	9.4 ± 2.0	9.6 ± 2.8
Tyr	3.1 ± 2.0	2.6 ± 1.5
Phe	1.5 ± 0.8	2.4 ± 1.4

[a] Mean and standard deviation values represent five matched pairs of bellies. [b] Chromatographically unresolved.

Table II. Amino Acid Composition of Raw and Fried Pork Belly Adipose Tissue

amino acids	concn,[a] μM/100 g			
	untreated		processed	
	raw	fried	raw	fried
Asp	2.9 ± 0.6	0.8 ± 0.2	3.1 ± 0.8	0.9 ± 0.4
Thr	37.3 ± 4.6	4.0 ± 0.8	42.9 ± 4.4	1.1 ± 0.5
Ser	25.7 ± 2.8	8.3 ± 0.8	30.3 ± 1.9	1.7 ± 0.2
Glu	23.6 ± 5.2	3.5 ± 0.9	25.6 ± 6.3	1.3 ± 0.3
Pro	9.4 ± 2.0	7.8 ± 1.0	13.0 ± 1.0	2.9 ± 0.7
Gly	35.0 ± 3.8	25.2 ± 13.8	53.2 ± 5.7	7.4 ± 2.0
Ala	55.7 ± 1.9	68.3 ± 7.0	58.5 ± 2.9	17.7 ± 1.1
Val	10.4 ± 3.1	6.2 ± 0.4	12.9 ± 1.3	3.0 ± 0.8
Met	3.0 ± 0.6	3.0 ± 0.5	3.3 ± 0.5	0.5 ± 0.2
Ile	5.2 ± 1.3	3.6 ± 2.2	5.1 ± 1.0	3.2 ± 2.3
Leu	12.0 ± 2.2	9.0 ± 1.2	9.8 ± 3.9	1.3 ± 0.8
Tyr	10.4 ± 0.9	3.6 ± 1.4	10.8 ± 0.9	0.8 ± 0.3
Phe	5.2 ± 1.1	4.6 ± 1.4	6.6 ± 1.3	0.2 ± 0.1

[a] Mean and standard deviation values represent three matched pairs of bellies.

RESULTS AND DISCUSSION

The amino acid composition of the adipose tissue of five matched pairs of bacon bellies, one side processed and the other left untreated, is shown in Table I. Comparison tests indicated significant increases ($P < 0.05$) in the concentrations of proline (52%) and glycine (41%) occur as a result of processing. Although there appears to be a major increase in glutamic acid concentration, this is the result of only one set among the five pairs. Minor variations occur in the concentrations of the other free amino acids. When bacon is fried, the high temperature (in the order of 350 °F) could either increase the free amino acid pool by protein degradation or cause loss in the existing pool as a result of thermal reactions. The adipose tissue from three additional pairs of bellies were analyzed before and after being fried; one belly of each pair was processed.

The concentration of amino acids in the adipose tissue of raw and processed bellies prior to being fried (Table II) are generally comparable to those reported in Table I, with the exception of glutamic acid concentration. After being fried, the green adipose tissues register losses of amino acids as high as 85 and 89% for glutamic acid and threonine, respectively, but for the most part, decreases are more moderate and in the order of 25–40%. Frying the processed samples, however, results in decreases in amino acid concentration of 70% or more for all amino acids except isoleucine. Differences between the amino acid concentrations in the processed and untreated samples are considerably greater after frying than before, emphasizing the

Table III. Proline, Glycine, and Nitrosamines from Adipose Tissue of Green and Processed Pork Bellies[a]

matched pair	treatment	Pro,[b] ppm	NPYR,[c] ppb	Gly, ppm	NDMA,[c] ppb
1	none	9.7	3	27.1	17
	proc	13.6	8	42.2	41
2	none	8.9	8	35.3	14
	proc	13.0	45	41.0	112
3	none	8.1	8	32.9	16
	proc	12.0	3	38.9	16
4	none	8.7	20	29.6	32
	proc	15.9	20	53.4	nd[d]
5	none	10.6	nd	27.4	nd
	proc	16.0	17	36.5	12
6	none	8.3	56	23.8	44
	proc	13.7	68	39.6	68
7	none	12.8	55	25.7	32
	proc	15.4	55	35.9	21
8	none	11.5	84	29.4	36
	proc	15.9	76	44.3	60

[a] All NA values confirmed by mass spectrometry. Values of matched pairs 1–5 represent results obtained in conjunction with Table I and pairs 6–8 correspond to results in Table II. [b] Amino acid available in tissue prior to frying. [c] NAs after frying. Nitrite was added to all samples prior to frying. [d] None detected.

effect of cure salts and heat on the stability of the amino acids in these adipose tissues. Proline, one of the amino acids suggested as a precursor for NPYR, presumably forms from the adipose tissue of bacon on frying. The concentration of proline, as well as that of glycine, increases significantly following processing, suggesting their formation as a result of the decomposition of collagen during processing. The proline concentrations in the adipose tissue of all eight pairs of bellies, processed and untreated, and the NPYR concentrations formed during frying with 100 mg of $NaNO_2$ are shown in Table III. There is no correlation between the concentration of proline in the unfried material and the concentration of NPYR formed on heating. The addition of nitrite to the untreated tissue led to the formation, in some instances, of as much as or more NPYR than appeared in the processed matched sample. It is interesting to note that the last three matched pairs of bellies (6–8 in Table III), which were used in the experiment detailed in Table II, produced very high levels of NPYR. No known variations in experimental procedure could have produced the additional nitrosamine. Nitrosodimethylamine was also observed in most of the fried adipose tissue samples. Since this nitrosamine has been detected during the pyrolysis of glycine (Ender and Ceh, 1971), the relationship of glycine to NDMA is also shown in Table III. The concentrations of NDMA were highly variable, ranging from none detected to 44 ppb in the untreated tissue and to 112 ppb in processed tissue. There was no correlation between the concentration of glycine in the tissue and the amount of NDMA nor between the concentrations of NDMA and NPYR formed in each of the samples. In general, however, the processed adipose tissue appeared to give rise to higher concentrations of NAs than did the untreated tissues, possibly because they were exposed to greater concentrations of sodium nitrite, 120 ppm in the cure mix plus 1000 ppm prior to being fried, whereas untreated tissues received only 1000 ppm sodium nitrite.

We also analyzed commercially produced "Country Cured" bacons because they are prepared without sodium ascorbate and thereby more closely resemble the bacons we studied in this investigation. Amino acid concentrations were determined after the intact, lean, and adipose tissues were fried, with the results summarized in Table IV. Comparison of these data with the results from the analysis of 18 raw untreated bellies (Table V) shows that raw adipose tissue accounts for much less of the free amino acid content than does either the raw intact or lean tissue. As a result of frying, the amino acid concentration in the intact and lean tissue was many-fold greater than in the adipose tissue.

Table IV. Amino Acid Concentrations in Fried Commercial "Country Cured" Bacon

amino acid	concn,[a] μM/100 g tissue: intact	lean	adipose
Asp	70.8	142.0	7.8
Thr	116.7	262.9	9.3
Ser	183.8	612.8	17.3
Glu	227.9	295.2	9.2
Pro	119.0	280.4	1.0
Gly	251.8	540.4	32.8
Ala	382.2	596.8	34.9
Val	134.0	316.8	15.6
Met	26.2	89.4	1.4
Ile	79.1	220.2	5.6
Leu	141.1	369.4	7.3
Tyr	51.4	129.2	1.7
Phe	66.4	137.4	2.5

[a] Average value of three bacons.

Table V. Amino Acid Concentrations in Untreated Pork Bellies

amino acid	concn,[a] μM/100 g tissue: intact	lean	adipose
Asp	18.9	26.3	2.5
Thr	24.3	26.7	27.2
Ser	36.0	31.7	25.7
Glu	26.5	33.5	10.6
Pro	14.9	23.9	6.9
Gly	109.0	148.4	34.8
Ala	170.9	199.1	46.2
Val	17.6	167.1	8.1
Met	4.2	5.7	1.7
Ile	9.9	14.5	3.7
Leu	16.9	23.0	8.2
Tyr	7.2	11.1	5.4
Phe	8.1	11.1	3.1

[a] Average value of 18 untreated bellies.

From the data presented in this paper, statistical analysis (comparison tests and analysis of variance techniques) of the differences in amino acid content before and after frying and NA concentration found after frying showed no significant correlation. Contrary to what has recently been reported (Bharucha et al., 1979; Coleman, 1978; and Hwang and Rosen, 1976), our data indicate that the NDMA and NPYR contents of fried bacon are independent of the concentration of free amino acids. Nitrosamine concentration was not significantly affected when the amino acid source, including proline, was decreased nor when the availability of free amino acids increased significantly. In conclusion, although free amino acids may play some role in NA formation in fried bacon, we think that they are not the limiting factor and that other substances and/or conditions present in the adipose tissue are responsible for the quantities of NAs formed in bacon.

(Precautions should be exercised in the handling of nitrosamines since they are potential carcinogens.)

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